Evaluation of molecular markers for discriminating *Gonatocerus morrilli*

A biological control agent imported from the origin of the glassy-winged sharpshooter

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Background and linear approach to biological control

1. We determined the origin [Texas (TX)] of GWSS that invaded California (CA) (de León et al. 2003, 2004a; Smith 2005). Very important accomplishment!!

Why?

Natural enemies have usually co-evolved with the target pest in the area of origin and therefore, have highly specialized host-finding abilities that may increase the potential success of a biological control program

(Narang et al. 1993; Scheffer & Grissell 2003; Brown 2004; Roderick 2004)

Background and linear approach to biological control

1. We determined the origin [Texas (TX)] of GWSS that invaded California (CA) (de León et al. 2003, 2004a; Smith 2005). Very important accomplishment!!

2. Initially, pops. of *G. morrilli* from TX & CA were identified as a single species (Phillips et al. 2001).

3. *G. morrilli* imported from TX had supposedly been released in CA since 2001.

4. But it was difficult to distinguish between native (CA) and imported (TX) natural enemies to determine their establishment or success.

5. We developed molecular markers that discriminated the native and imported natural enemies (de León et al. 2004b,c). Very important accompl.!!

6. From morphological, molecular, and hybridization studies we discovered that pop. from CA was actually a new species, *G. walkerjonesi* S. Triapitsyn (de León et al. 2004b,c,d, 2006; Triapitsyn 2006). Very important accompl.!!

Objective

To evaluate the utility of various types of molecular markers in detecting and discriminating *G. morrilli* populations released in California against GWSS

We tested two previously developed markers:

ISSR-PCR DNA fingerprinting banding patterns
Amplification size of the ITS2 rDNA fragment

and

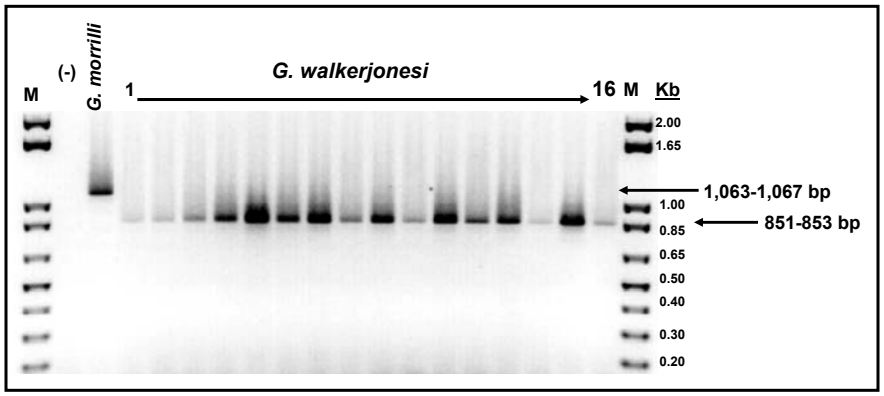
Two newly developed 'one-step' species-specific markers targeting the ITS2 region of *G. morrilli* and *G. walkerjonesi*

Summary of the '*G. morrilli*' release and collection sites in California

Location Code	City	County	No. Ind.
2002-2004			
OSJC1	San Juan Cap.	Orange	94
RGIV1	Glen Ivy	Riverside	31
DPAU1/2	Pauma	San Diego	99
RTEM1/2	Temecula	Riverside	31
DSMA1	San Marcos	San Diego	72
RUCR1	UC Riverside	Riverside	9
ACAP1	Pomona	Los Angeles	3
RVAN2	Van Burea	Riverside	6
RGDR1	Myers St.	Riverside	14
BHEL1	Hellman	San Bernardino	3
2005			
RGIV1	Glen Ivy	Riverside	2
VSTO1	Moorpark	Ventura	4
VDUD1/2	Fillmore	Ventura	3
VBART1	Fillmore	Ventura	4
DPAU1/2	Pauma	San Diego	5
RMCM1	Hemet	Riverside	2
2006			
RGIV1	Glen Ivy	Riverside	2
RCOD1	Palm Desert	Riverside	2
DSMA1	San Marcos	San Diego	6
DPAU1/2	Pauma	San Diego	4
OMVJ1	San Juan Cap.	Orange	11
OSJC1	San Juan Cap.	Orange	2
OIRH1	Irvine	Orange	2
RUCR1	UC Riverside	Riverside	2

In total, 329 specimens from collections stored in ethanol were included from 19 locations. Specimens collected throughout the year per location were pooled.

Fig. 1. Example of the utility of the size of the ITS2 fragment.



Included are 16 randomly chosen post-release specimens from San Juan Capistrano

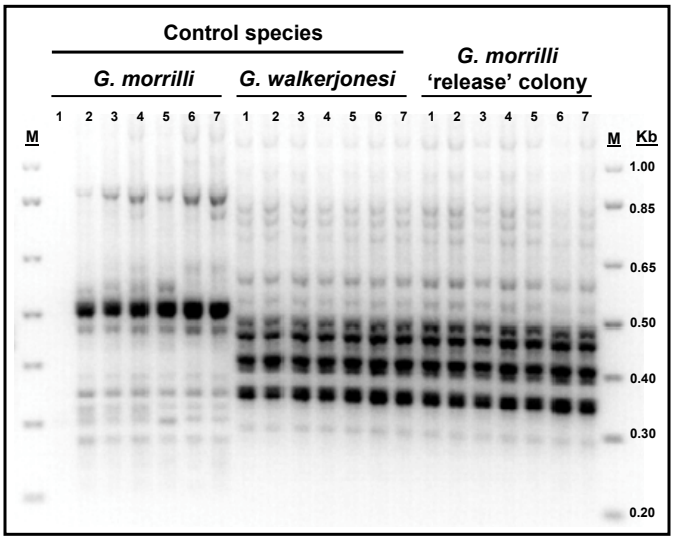
Post-release specimens discriminated by size of ITS2 rDNA fragment

Location Code	City	County	No. Ind.	#ITS2/ Gwj	#ITS2/ Gm
2002-2004					
OSJC1	San Juan Cap.	Orange	94	94	0
RGIV1	Glen Ivy	Riverside	31	31	0
DPAU1/2	Pauma	San Diego	99	99	0
RTEM1/2	Temecula	Riverside	31	31	0
DSMA1	San Marcos	San Diego	72	72	0
RUCR1	UC Riverside	Riverside	9	9	0
ACAP1	Pomona	Los Angeles	3	3	0
RVAN2	Van Burea	Riverside	6	6	0
RGDR1	Myers St.	Riverside	14	14	0
BHEL1	Hellman	San Bernardino	3	3	0
2005					
RGIV1	Glen Ivy	Riverside	2	0	2
VSTO1	Moorpark	Ventura	4	4	0
VDUD1/2	Fillmore	Ventura	3	3	0
VBART1	Fillmore	Ventura	4	4	0
DPAU1/2	Pauma	San Diego	5	5	0
RMCM1	Hemet	Riverside	2	2	0
2006					
RGIV1	Glen Ivy	Riverside	2	0	2
RCOD1	Palm Desert	Riverside	2	0	2
DSMA1	San Marcos	San Diego	6	6	0
DPAU1/2	Pauma	San Diego	4	4	0
OMVJ1	San Juan Cap.	Orange	11	11	0
OSJC1	San Juan Cap.	Orange	2	2	0
OIRH1	Irvine	Orange	2	2	0
RUCR1	UC Riverside	Riverside	2	2	0

Started with 2002-2003 collections (280 Ind.)

In total, 329 specimens from collections stored in ethanol were included from 19 locations. Specimens collected throughout the year per location were pooled.

Fig. 2. Evaluation of the original *G. morrilli* colony that was used for releases in California by the ISSR-PCR DNA fingerprinting method



After this, I sent *G. morrilli* from TX, origin of GWSS, to CDFA (& UC-Riverside). CDFA started new releases of *G. morrilli* colony in the summer of 2005.

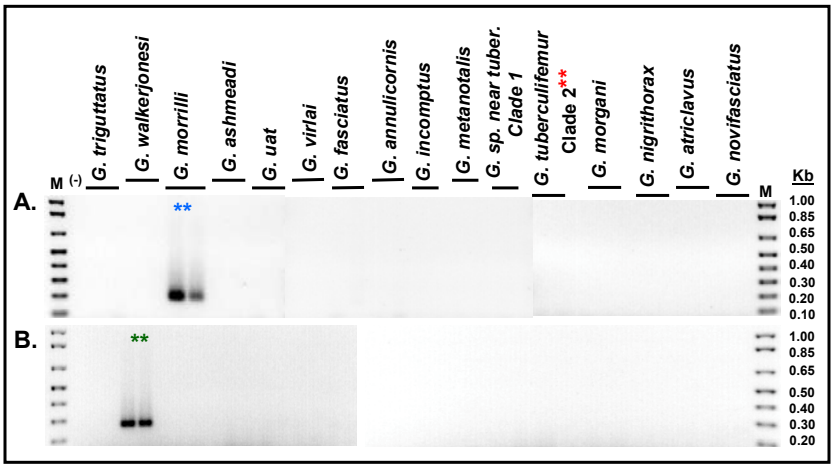
Post-release specimens discriminated by size of ITS2 rDNA fragment

Location Code	City	County	No. Ind.	#ITS2/ Gwj	#ITS2/ Gm	
2002-2004						
OSJC1	San Juan Cap.	Orange	94	94	0	
RGIV1	Glen Ivy	Riverside	31	31	0	
DPAU1/2	Pauma	San Diego	99	99	0	
RTEM1/2	Temecula	Riverside	31	31	0	
DSMA1	San Marcos	San Diego	72	72	0	
RUCR1	UC Riverside	Riverside	9	9	0	
ACAP1	Pomona	Los Angeles	3	3	0	
RVAN2	Van Burea	Riverside	6	6	0	
RGDR1	Myers St.	Riverside	14	14	0	
BHEL1	Hellman	San Bernardino	3	3	0	
2005						
RGIV1	Glen Ivy	Riverside	2	0	2	Fall, 2005
VSTO1	Moorpark	Ventura	4	4	0	
VDUD1/2	Fillmore	Ventura	3	3	0	
VBART1	Fillmore	Ventura	4	4	0	
DPAU1/2	Pauma	San Diego	5	5	0	
RMCM1	Hemet	Riverside	2	2	0	
2006						
RGIV1	Glen Ivy	Riverside	2	0	2	Spring, 2006
RCOD1	Palm Desert	Riverside	2	0	2	Fall, 2006
DSMA1	San Marcos	San Diego	6	6	0	
DPAU1/2	Pauma	San Diego	4	4	0	
OMVJ1	San Juan Cap.	Orange	11	11	0	
OSJC1	San Juan Cap.	Orange	2	2	0	
OIRH1	Irvine	Orange	2	2	0	
RUCR1	UC Riverside	Riverside	2	2	0	

Continued with 2004-2006 collections

In total, 329 specimens from collections stored in ethanol were included from 19 locations. Specimens collected throughout the year per location were pooled.

Fig. 3. Cross-reactivity assays using the 'one-step' species-specific ITS2 diagnostic markers. 16 *Gonatocerus* Nees species from N., S. America.



A. gmtx (*G. morrilli*) (204-bp) and B. wjca (*G. walkerjonesi*) (249-bp)
**Being described as a new species by S. Triapitsyn

Fig. 4. Molecular analysis of the original *G. morrilli* 'release' colony used before Summer '05 with the 'one-step' species-specific ITS2 markers.

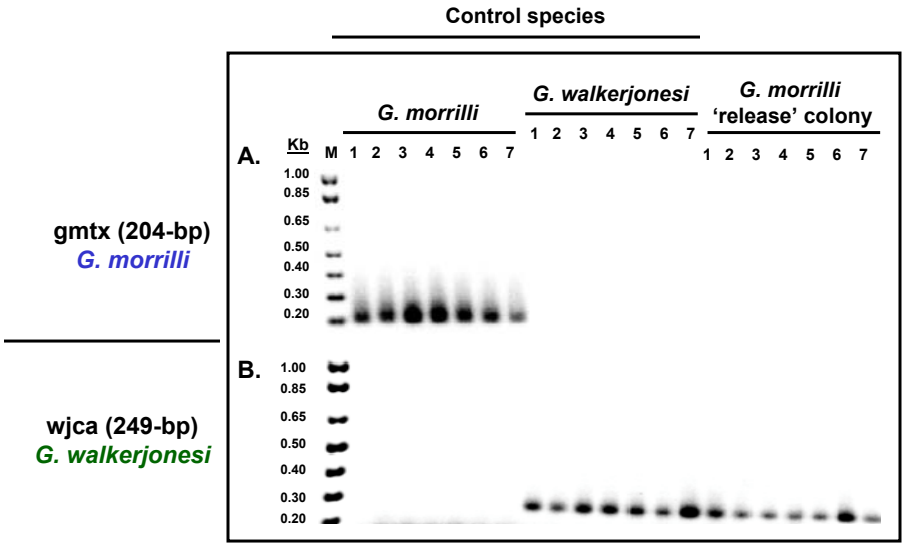
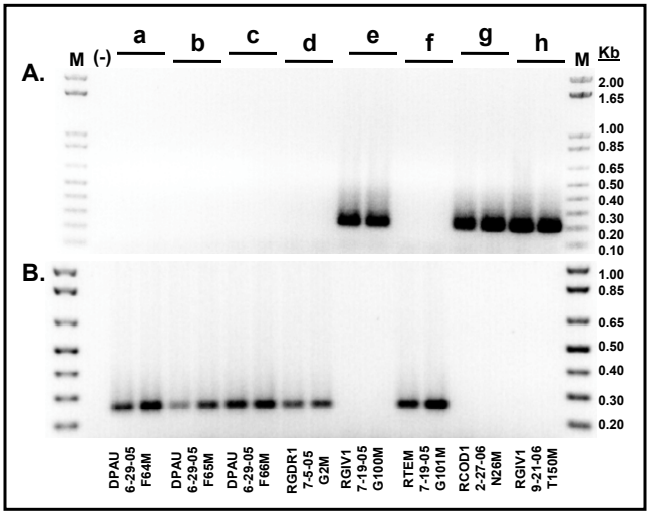


Fig. 5. Example of the utility of the 'one-step' ITS2 markers. We randomly screened post-release specimens, including those that previously tested positive using the ITS2 fragment size.

gmtx (204-bp)
G. morrilli

wjca (249-bp)
G. walkerjonesi



Summary

1. Results confirm the utility of the molecular markers in monitoring the establishment of *G. morrilli* or the success of the BC program.

2. Through our molecular research approach, we turned the biological control program around, toward the right direction.

3. Good recovery rates are now being seen with *G. morrilli* in certain regions of California; confirming the importance of 'origin' in BC programs! (Another issue to consider is 'Climate Matching').

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